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Research Report
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Age estimation using the ratio of dental pulp to tooth volume by CT scan in dogs

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ABSTRACT

Importance: Accurate age estimations in dogs are essential for various veterinary and clinical applications. Traditional methods based on dental appearance are error-prone because of external influences, necessitating the development of more precise methodologies.
Objective: This study examined the association between chronological age and pulp to tooth (P/T) volume ratio in dogs using computed tomography (CT) of the right maxillary canine and right maxillary fourth premolar (PM4) teeth.
Methods: Ninety-five canine skulls, aged five to 216 months, were examined using a two-slice CT scan machine. Volumetric analyses of the right maxillary canine and PM4 were performed. Statistical analysis, including Spearman's correlation and regression analysis, was conducted to assess the relationship between age and the P/T volume ratio.
Results: This study included 60 male and 35 female dogs, with no significant age difference between genders ($p = 0.655$). A strong negative correlation was observed between age and the P/T volume ratio for the right maxillary canine ($r = 0.88$, $p < 0.001$) and PM4 ($r = 0.77$, $p < 0.001$) teeth. Logarithmic regression models provided a better fit for age prediction ($R^2 = 0.78$ for the right maxillary canine, $R^2 = 0.67$ for PM4) compared to linear models.
Conclusions and Relevance: CT-based volumetric analysis of the P/T ratio in the right maxillary canine and PM4 teeth offers a precise, non-invasive method for estimating the age of dogs. This technique can improve the accuracy of age estimations, particularly in forensic and clinical settings, providing valuable insights for veterinarians and researchers.

Keywords: Age factors; dog; computed tomography angiography; volumetric computed tomography; teeth

INTRODUCTION

Assessments of internal dental structures, such as dental pulp, are considered one of the most reliable methods for age estimations because it is less influenced by external factors such as nutrition, hormonal changes, and environmental conditions [1-3]. The anatomy of teeth in dogs and humans comprises various structures, including the crown, root, surrounding tissues, and multiple layers, such as enamel, dentin, cementum, and dental pulp [4,5]. Teeth constitute the hardest tissue in humans and dogs [6]. In humans, the enamel

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Conflict of Interest
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comprises 95% hydroxyapatite crystals and 5% organic matter and water by weight, making it the most mineralized structure in the body that is highly resistant to environmental changes even after death [7,8]. When considering weight proportions, dentin is less mineralized than enamel, constituting approximately 96% by weight. On the other hand, it surpasses the mineralization levels of bone and cementum, accounting for approximately 65% in weight [8]. The apical canal of a tooth is the narrow passage located at the root tip, through which the nerves and blood vessels of the tooth enter and exit, playing a crucial role in maintaining the vitality and health of the tooth. In particular, dogs have a more complex apical delta than humans, with multiple small channels branching out at the root apex [9].

Several methods are used to estimate age in humans. One method is investigating tooth eruption and growth, which is a reliable factor in age estimations [10,11]. The consequence of tooth eruption and tooth appearance can also be used to predict the age of dogs [12]. On the other hand, relying solely on the visual characteristics of teeth for age estimation is unreliable for age verification in legal contexts [13], underscoring the need to develop a more precise methodology.

Brunescence, or lens browning, is another method for estimating the age of dogs. In this examination, brunescence increases with age, with higher light absorption in the nucleus compared to the cortex, giving the lens a brown appearance. Hence, brunescence might be useful as a predictor of the age of dogs [14]. Another examination to predict a dog's age involves assessing the refractivity of the ocular crystalline lens. As animals age, increased refractivity causes the two pinpoint lens reflections in the eyes of young adult dogs and cats to appear larger. These changes can be categorized to divide the lifespan into age groups, allowing the age of mature dogs and cats with unknown histories to be determined more accurately than with the dental method of age estimation [15]. Nevertheless, these methods are unsuitable in instances involving deceased animals and discovered skulls lacking documented provenance.

After a tooth erupts through the gingiva, the secretion of secondary dentin initiates within the tooth, leading to a decrease in the internal space of the tooth pulp with age, which can be used as a precise method for estimating the age of humans [16,17].

The predominant dental age estimation techniques often involve tooth extraction, with some requiring microscopic sections from at least one tooth [17,18]. These methods can be applied in postmortem studies but are not clinically viable for routine use in living animals or those with uncertain histories. Radiographic images were initially used to assess the tooth length-to-width ratio in relation to dental pulp as a non-invasive method in humans [19,20]. On the other hand, radiography is constrained by the superimposition of the surrounding structures.

Three-dimensional and two-dimensional images can be used to examine the size and volume of teeth and the surrounding structures without the superimposition of different tissue structures [21]. Computed tomography (CT) scans can assess the overall oral environment, but the volume of teeth and dental pulp can also be calculated accurately. Therefore, currently, this method is considered superior to dental radiography.

In light of recent studies on human age estimation using dental volume and the significant correlation observed between these two factors, this relationship will be addressed more prominently in dogs because of their shorter lifespan and the larger volume of teeth and dental pulp in canine species, suggesting increased precision and sensitivity in age estimations of dogs.

The literature on age estimation predominantly focuses on humans, with extensive research utilizing dental CT scans to determine age. On the other hand, studies on age estimations in animals, particularly dogs, are limited. To the best of the authors' knowledge, there are no reports on age estimations using CT scans in other animals. This highlights a significant gap in literature and underscores the novelty of this study.

This study aimed to establish a robust framework for age estimation using CT scans, applicable across various scenarios, including those involving deceased animals and undocumented specimens. This advance can enhance forensic analysis, improve veterinary practices, and contribute to the broader field of biological age assessment. This study provides a new approach to estimating the age of dogs and sets a precedent for future studies in veterinary science and other animal species.

METHODS

Study population

Ninety-five canine skulls, comprising 60 males and 35 females of various breeds, aged between five and 216 months, were included in this study. The data were collected from February 2022 to January 2024 from cases referred exclusively for skull evaluation to the School of Veterinary Medicine at the University of Tehran. A veterinary resident was responsible for collecting data from patients referred to the veterinary radiology department of the veterinary college with the request for a skull CT scan. This study only used permanent teeth. The inclusion criteria stipulated that animals must have been referred to for reasons unrelated to dental, jaw, and facial problems. They underwent a thorough physical examination and blood testing to exclude systemic problems. Furthermore, only dogs that had been consuming commercially available dry foods or homemade diets and had no history of chewing hard objects were included. Dogs with unclear dietary histories, oral or dental abnormalities before the dental treatments, or chewing habits involving hard objects were excluded. **Table 1** lists the breeds of the participating dogs and the corresponding counts for each breed. The mixed breed dogs were categorized into two groups based on their size: 'Mix S' (small mixed breeds weighing less than 10 kg) and 'Mix L' (large mixed breeds weighing more than 10 kg).

Anesthesia protocol

Anesthesia was induced using a combination of intravenous ketamine (5 mg/kg; Ketaminol 5%, Interchemie, Netherlands) and diazepam (0.28 mg/kg; Diazepam 10 mg/2 mL, Caspian Tamin Pharmaceutical, Iran). After induction, the animals were endotracheally intubated, and anesthesia was maintained with isoflurane inhalation (1.2%–1.5%; isoflurane USP, Piramal Critical Care, USA). The anesthetic depth was monitored carefully throughout the procedure using a pulse oximeter to assess the jaw tone, eyelid responsiveness, heart rate, respiratory rate, and peripheral oxygen saturation. Spontaneous ventilation was maintained for the entire duration of anesthesia. This general anesthesia protocol was used for most subjects. On the other hand, alternative drug combinations were occasionally used in cases where patient-specific considerations were required.

Imaging procedures

All CT examinations were performed using a 2-slice CT scan machine (Somatom-Spirit, Siemens, Germany) at the Department of Diagnostic Imaging in the Veterinary Faculty,

Table 1. Dog breeds participating in the study

Breed	Number
Shih Tzu	12
Mix S[a]	11
Spitz	8
Pitbull	8
German Shepherd	9
Mix L[b]	7
Terriers	10
Pomeranian	6
Chihuahua	1
Iraqi	2
Pointer	2
Samoyed	2
Pug	3
Husky	1
Bulldog	1
Boxer	1
Pekinese	1
Dalmatian	1
Maltese	1
Golden Retriever	1
Dachshund	1
Yorkshire Terrier	1
Chow Chow	1
Poodle	1
Kangal	1
Labrador Retriever	1
Boomi	1

[a]Mixed breed dogs weighing < 10 kg; [b]Mixed breed dogs weighing > 10 kg.

Tehran University, Tehran, Iran. A surgical resident administered anesthesia before the imaging procedures. Dogs were positioned in sternal recumbency for a CT scan examination of the skull. The standard skull CT scan protocol was applied, with the parameters set to 120 kVp, 60–65 mA, an acquisition slice thickness of 1 mm, and a reconstructed slice thickness of 1 mm. The dorsal and sagittal images were reconstructed to enhance the volume estimation of the right maxillary canine and maxillary fourth premolar (PM4) teeth.

Data analysis

All CT images were archived in the PACS system (Orthanc, an open-source PACS server) and saved as DICOM files. Volumetric analyses were conducted using three dimensional (3D) Slicer 5.2.2 (**Fig. 1**). A board-certified veterinary radiologist (M.H.) performed the evaluations in the Osteo window. Specific volumetric analysis techniques, including threshold-based segmentation, were used to assess the right maxillary canine and PM4 teeth. The features of the 3D Slicer used for the reconstruction and volume assessments included segmentation tools, volume rendering, and measurement modules. In this study, the pulp to tooth (P/T) ratio was defined as the ratio between the volume of the pulp cavity and the total tooth volume, including the combined volume of the hard tissues (enamel and dentin) and the pulp cavity of the tooth.

Statistical analysis

The sample population of healthy dogs (n = 95) was used to assess the correlation between age and the P/T volume ratio, considering gender. Statistical analysis was conducted using SPSS Statistics v29 software (IBM, USA).

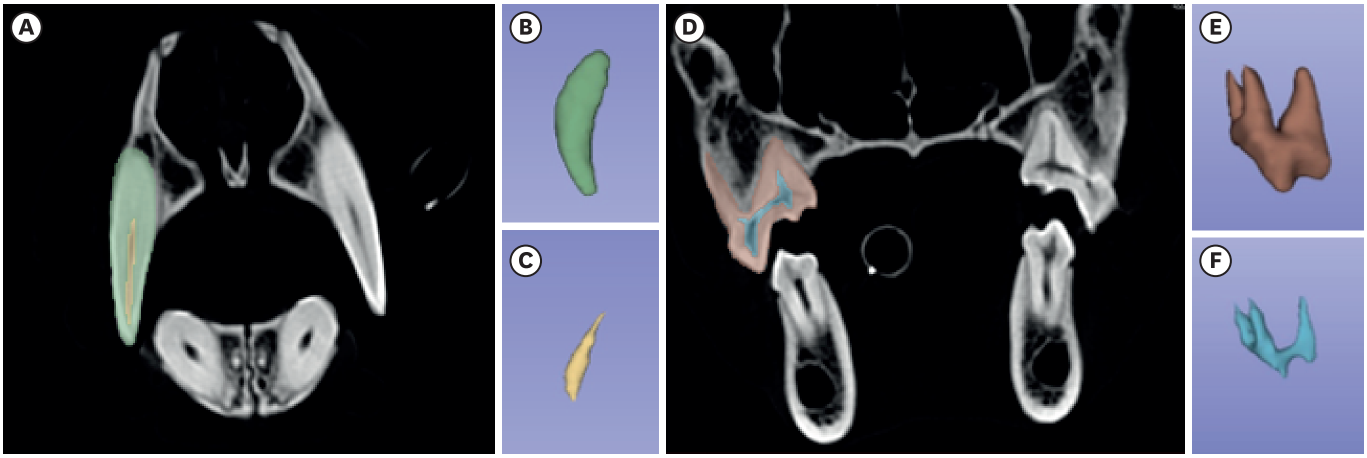

Fig. 1. The 3D volumetric models and CT images of right maxillary canine and PM4 teeth. Panels (A-C) show the right maxillary canine tooth: (A) manual delineation of the right maxillary canine tooth on the CT image. (B, C) 3D reconstructions of the tooth structure (B) and the corresponding pulp cavity (C) generated using 3D Slicer software. Panels (D-F) represent the right maxillary PM4 tooth: (D) manual delineation of the tooth, followed by (E, F) 3D reconstructions of the tooth structure (E) and corresponding pulp cavity (F) also produced using 3D Slicer software.
3D, three dimensional; CT, computed tomography; PM4, fourth premolar.

Descriptive analysis, including the computation of mean and standard deviation, was conducted to ascertain the age distribution across the entire animal population and within each gender subgroup. The mean and standard deviation calculations were conducted for the P/T volume ratio of each right maxillary canine and PM4 tooth individually.

Linear correlations were determined using the Spearman's rank correlation coefficient, calculated separately for each gender and tooth, to assess the relationship between age and the P/T volume ratio; p values < 0.001 were considered significant.

Linear and logarithmic regression analyses were carried out individually, considering the P/T volume ratio as the independent variable and age as the dependent variable for each right maxillary canine and PM4 tooth ($p < 0.001$). These analyses aimed to explore and model the relationship between age and the P/T volume ratio.

RESULTS

Descriptive statistics

Ninety-five dogs from various breeds were examined, with 35 (36.8%) and 60 (63.2%) being female and male, respectively. The mean age of the dogs was 44.37 ± 55.66 months. In particular, the mean ages of the female and male dogs were 43.89 ± 58.34 months and 44.97 ± 54.1 months, respectively. No statistically significant difference ($p = 0.655$) in the mean ages was noted between male and female dogs.

The mean ratio of the right maxillary canine P/T volume (in percentage) was 8.02 ± 10.18 and 7.7 ± 9.17 for male and female dogs, respectively. Statistical analysis revealed no significant difference ($p = 0.55$) in the mean ratio of the right maxillary canine P/T volume between male and female dogs. Similarly, the mean ratio of the PM4 P/T volume (in percentage) was 5.10 ± 9.41 and 5.14 ± 9.08 for male and female dogs, respectively. Upon analysis, no significant difference ($p = 0.766$) in the mean ratio of the pulp to the PM4 volume was observed between male and female dogs (**Table 2**).

Table 2. Comparison of the mean age, right maxillary canine, and PM4 P/T volume ratio (percentage) between male and female dogs

Parameters	Male	Female	*p* value
Age (mon)	44.97 ± 54.1	43.89 ± 58.34	0.655
Right maxillary canine P/T ratio	8.02 ± 10.18	7.7 ± 9.17	0.55
PM4 P/T ratio	5.1 ± 9.41	5.14 ± 9.08	0.766

Values are presented as mean ± standard deviation (%).
PM4, fourth premolar; P/T, pulp to tooth.

Table 3. Regression models for right maxillary canine and PM4 pulp to tooth volume

Tooth type	Model	Formula	R^2	95% CI
Right maxillary canine	Linear	y: 92.89 − 3.79x	0.45	−4.65, −2.93
	Logarithmic	y: 147.25 − 46.32 log (x)	0.78	−51.45, −41.18
Right maxillary PM4	Linear	y: 109.83 − 5.92x	0.47	−7.90, −4.42
	Logarithmic	y: 180.64 − 60.64 log (x)	0.67	−69.55, −51.73

PM4, fourth premolar; CI, confidence interval.

Correlation between age and P/T ratio

The Spearman's correlation coefficient between age and the ratio of the right maxillary canine P/T volume yielded a value of 0.88 ($r = 0.88$), indicating a strong and statistically significant negative correlation ($p < 0.001$). This correlation was slightly stronger in female dogs ($r = 0.90$) than male dogs ($r = 0.87$). Similarly, age showed a significant negative correlation with the pulp-to-PM4 volume ratio, with a coefficient of 0.77 ($p < 0.001$). The correlation coefficient was 0.84 and 0.73 for female and male dogs, respectively.

Regression analysis of the right maxillary canine P/T ratio to age

Regression analysis was conducted to explore the association between age (dependent variable, *y*) and the percentage of the right maxillary canine P/T volume (independent variable, *x*). The scatter plots were initially plotted to assess their relationship. Subsequently, linear and logarithmic regression models were applied to the dataset (**Table 3**).

The linear regression model revealed a significant negative relationship between age and the right maxillary canine P/T volume ratio ($p < 0.001$). The logarithmic model fitted the data better based on the adjusted R^2 values (**Fig. 2A**).

Regression analysis of the right maxillary PM4 tooth P/T ratio to age

Regression analysis was performed to examine the correlation between age (dependent variable, *y*) and the percentage of the right maxillary PM4 P/T volume (independent variable, *x*). Linear and logarithmic regression models were applied to the dataset (**Table 3**). The linear regression model indicated a significant negative relationship between age and the right maxillary PM4 P/T volume ratio ($p < 0.001$). Similarly, the logarithmic regression model showed a comparable negative relationship. On the other hand, the logarithmic model provided a better fit to the data based on adjusted R^2 values (**Fig. 2B**).

DISCUSSION

This study examined the association between chronological age and the P/T volume ratio in the maxillary right canine and PM4 teeth of dogs. Many diseases in dogs, including malignancies and metabolic disorders, such as Cushing's syndrome and thyroid diseases, vary in prevalence and onset across different age groups. In addition, an age estimation based solely on dental appearance entails significant errors because it can be influenced by

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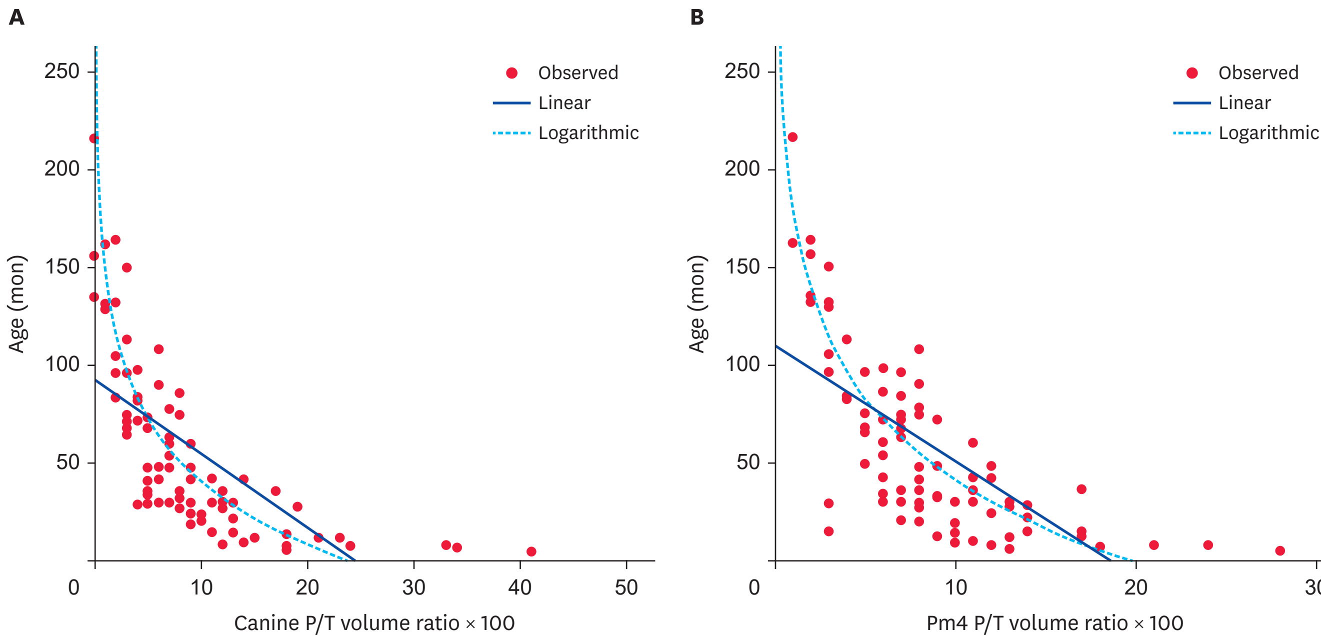

Fig. 2. Logarithmic regression relationships between chronological age and P/T volume ratios of right maxillary canine (A), with the formula: $y = 147.25 - 46.32 \log(x)$, $R^2 = 0.78$ and PM4 (B) with the formula: $y = 180.64 - 60.64 \log(x)$, $R^2 = 0.67$.
PM4, fourth premolar; P/T, pulp to tooth.

factors such as dietary intake, treatment methods like professional dental cleaning, and the condition of the teeth, which may be affected by regular dental care practices, the type of housing (indoor vs. outdoor), and whether the dog is a service animal [22-24].

With age, the space of the dental pulp canal decreases and becomes narrower. The changes in the pulp cavity volume coincide with secondary dentin deposition, a natural morphological change linked with the aging process [25,26]. Tertiary dentin, or reparative dentin, is formed in response to external stimuli such as injury, caries, or therapeutic procedures. This type of dentin is less organized and typically localized to an injury site, serving as a protective barrier for the dental pulp [27]. Secondary dentin is continuously produced with age, narrowing the pulp cavity in intact teeth and gradually reducing the pulp cavity volume. Consequently, a decreased pulp cavity volume in intact teeth can be a reliable predictor of dental age [25]. Gustafson introduced the secretion and replacement of secondary dentin as one of the six physiological factors for age estimation in humans, along with attrition, periodontal disease, cementum apposition, root resorption, and root transparency. In 1950, he developed a regression formula to estimate age based on these factors [17].

Various non-invasive radiography studies have been conducted to determine the linear relationship between the tooth-to-pulp ratio for age estimation in humans [19,28-31]. On the other hand, the overlapping of various structures, the inability to examine pulp alterations, and the overall shape of the tooth represent the primary limitations of these studies.

The limitation of radiography was overcome using CT scan imaging to measure the ratio of pulp volume to the right maxillary canine tooth volume and pulp volume to the right maxillary PM4 tooth volume as methods to estimate the chronological age of dogs. Although the maxillary canine and maxillary PM4 teeth are more prone to fracture [32-34], this study focused on the right maxillary canine and PM4 teeth because of their larger volume compared to other teeth.

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Several studies have reported a negative correlation between age and the P/T ratio measured by CT [1,25,35-38]. Despite the numerous differences between the human and canine anatomy and physiology, the regression analysis results from humans have consistently shown a persistent inverse association between age and the P/T ratio, mirroring the findings observed in the present study.

Several human studies have consistently shown a significant correlation between age and the P/T volume ratio across both genders [1,25,35-44]. This study also revealed a robust and significant relationship between age and the P/T volume ratio in the right maxillary canine and PM4 teeth. In particular, this correlation was slightly stronger in females than males. These findings concur with those reported in previous human studies [21,39,41].

Various tooth volume analyses, encompassing different types of teeth, were conducted across several studies to predict age in humans. These include assessments of the maxillary central incisor [25,35,42,45], mandibular first premolar [25,35,38,42], mandibular canine [25,35,36,42,43], mandibular second premolar [35], and maxillary canine [36,43,44]. These studies explored the correlation between the P/T volume ratio and age.

The right maxillary canine and PM4 teeth were assessed in various breeds of dogs. The P/T volume ratios of the right maxillary canine and PM4 teeth exhibited a robust negative correlation with age. On the other hand, this analysis showed that the correlation coefficients for the right maxillary canine ($R = 0.88$) and the right maxillary PM4 ($R = 0.87$) were similar. This small difference suggests that both teeth have strong correlations with age, and the difference in their correlation strengths was not significant.

In this study, the logarithmic model showed superior predictive accuracy to the linear model, particularly its ability to account for the nonlinear aspects of age-related changes in tooth development. This enhanced fit underscores the robustness of the logarithmic model in capturing the complexity of biological growth patterns, which are often nonlinear.

In studies involving various animal species, researchers have explored age estimation techniques using the tooth width to pulp width ratio. Park et al. [46] conducted a radiographic analysis to estimate age using the ratio of the right maxillary canine tooth width to pulp width in cats. Their study revealed a significant relationship ($R^2 = 0.92$) between age progression and decreased tooth width-to-pulp width ratio [46]. This finding aligns with the observations in dogs. In particular, this ratio exhibited similar significant meaning across male and female feline specimens, which is consistent with a study conducted on coyotes [47].

CT scans were used to estimate the age of dogs using the pulp volume to tooth volume ratio. In addition to the right maxillary canine tooth, the right maxillary PM4 tooth was also included in the analysis. Although previous studies, such as Pereira et al. [48], used radiography for age estimations using the carnassial tooth, these findings represent a significant addition using CT scans for this purpose.

Conventional CT was used because of its ability to provide detailed volumetric analysis and its broader availability in veterinary radiology compared to cone-beam CT (CBCT). Although CT requires longer anesthesia and is often located outside the dental operating room, it offers several advantages over conventional radiography. Although conventional radiography is more accessible and cost-effective in routine veterinary practice, CT provides superior 3D visualization and detailed imaging of soft and hard tissues, which is crucial for accurate age estimations based on P/T volume analysis. Despite the practical challenges, the enhanced accuracy of age determination through CT imaging justifies its use in this context.

Although this study provided valuable insights, it is essential to acknowledge some limitations. The sample size and breed variability in this study may have influenced the results. Further research with larger and more diverse populations is warranted to validate these findings. In addition, using 1 mm slice thickness with medical CT may not have provided the optimal resolution for small dogs. Future studies may benefit from using CBCT, which is commercially available and provides narrower sections, enhancing the imaging of hard tissues. Furthermore, exploring other teeth and imaging modalities could improve the accuracy and reliability of canine age estimation techniques.

Another limitation of this study is that, while dogs with a history of chewing hard objects were excluded, this study did not account for other factors related to the chewing force, which can cause tooth wear and affect the production of tertiary dentin by odontoblasts, contributing to pulp cavity narrowing [49]. Future studies should group dogs based on breed size, dietary habits, and chewing behavior to control for these factors, ensuring a more accurate assessment of age estimation variables.

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